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## The peroxisome proliferation machinery in yeast

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## Summary

Cellular life comprises of three domains: prokaryotes, archaea and eukaryotes. The presence of a nucleus that contains DNA and specific membrane-bound structures called organelles is a unique feature of eukaryotic cells. These intracellular structures are very dynamic and exhibit a characteristic morphology and architecture that is distinct to every organelle. Organelle biogenesis is the process by which new organelles are formed and is one of the fundamental topics in contemporary cell biology. The mechanisms of organelle formation are highly conserved among different species. Organelles can be grouped into two categories as autonomous and non-autonomous. ER, mitochondria, chloroplasts belong to the first category and Golgi, secretory vesicles and the vacuole to the second.

A relatively recently identified sub-cellular organelle is the peroxisome. Peroxisomes are ubiquitously present, highly dynamic organelles whose number, size and function are vastly dependent on the cell type and growth conditions. For instance, they are involved in methanol metabolism in yeast, photorespiration in plants, and penicillin biosynthesis in *Penicillium chrysogenum*. However, they share two generalized functions:  $\beta$ -oxidation of fatty acids and  $H_2O_2$  metabolism. The significance of these organelles is exemplified by the identification of several human inherited diseases, caused due to dysfunction of peroxisomes.

Yeast is an attractive system in which several fundamental cellular mechanisms can be successfully investigated and, because of the degree of conservation, the knowledge obtained can be related to higher eukaryotes like man. The size and number of peroxisomes in yeast can be precisely prescribed by manipulation of the growth conditions. Glucose grown yeast cells contain one or few peroxisomes per cell. But upon shifting these cells to media containing methanol or oleic acid peroxisome proliferation is induced. This is because of the induction of the synthesis of enzymes required for the catabolism of these compounds.

Unlike mitochondria and chloroplasts, which are known to be autonomous organelles, the biogenesis of peroxisomes is still highly debated. The classical idea of peroxisome biogenesis was by the growth and division model, which suggested that new peroxisomes are formed by the division of pre-existing ones. This process is coupled to a strictly regulated inheritance mechanism to ensure every cell has at least one organelle. This model was however challenged by the observation that peroxisomes can be formed from the ER as well. However, the suggestion that peroxisomes invariably arise from the ER is not widely

accepted. The main reason for this is that formation of peroxisomes from ER is so far only observed in mutant cells lacking peroxisomes and not in wild type cells. On the other hand several proteins required for fission and inheritance of peroxisomes are now identified in different organisms.

Molecular mechanisms of peroxisome fission in yeast (*Saccharomyces cerevisiae* and *Hansenula polymorpha*) are the topic of this study. The question if peroxisomes are formed from fission of pre-existing ones or whether they are formed from ER or if both these pathways co-exist is also addressed in this thesis.

Chapter 1 presents the overview of the current knowledge of peroxisome fission. The components of the molecular machinery involved in this process are described in detail.

In chapter 2 peroxisome fission in *S. cerevisiae* was analyzed. In this fission process the function of so called dynamin-like proteins (DLPs) is essential. Yeast contain three DLPs (Vps1, Dnm1 and Mgm1), which play a role in membrane fission and fusion processes. Of these, Vps1 was initially identified as a protein involved in vacuolar protein sorting, Dnm1 as a protein responsible for mitochondrial fission whereas Mgm1 as a protein required for mitochondrial fusion. Absence of Vps1 leads to a remarkable reduction of peroxisome numbers; however peroxisome proliferation was not completely blocked. The first research question was to investigate whether Dnm1 was responsible for this residual fission effect. To this end, *S. cerevisiae* cells lacking Dnm1 (*dnm1* cells) were analyzed using fluorescence microscopy. Peroxisome numbers were not altered in glucose-grown *dnm1* cells, but a very clear reduction in organelle numbers was observed when cells were grown on oleate, a carbon source that induces peroxisome proliferation. Similar observations were made in cells lacking the membrane protein Fis1, a protein that was known to recruit Dnm1 to mitochondria. Unexpectedly, detailed fluorescence microscopy of cells producing Dnm1-GFP or GFP-Fis1 demonstrated that both proteins have a dual localization on mitochondria and peroxisomes.

Cells that lack both Vps1 and Dnm1 (*vps1.dnm1* cells) generally contained a single peroxisome. Time lapse imaging indicated that during budding of these cells this peroxisome formed long protrusions into the developing bud. This organelle divided at a very late stage of the budding process, possibly during cytokinesis. These results clearly show that both the DLPs Vps1 and Dnm1 are required for peroxisome fission in *S. cerevisiae*.

A second yeast model used in this research is *H. polymorpha*. *H. polymorpha* can use methanol as a sole carbon source for growth. In this organism peroxisome numbers can readily be prescribed by manipulation of the growth conditions. This property renders it an attractive model organism to study the proliferation machinery of peroxisomes.

Chapter 3 demonstrates that in *H. polymorpha* peroxisome fission is the major process of organelle multiplication. Moreover, unlike in *S. cerevisiae*, deletion of *VPS1* had no effect on peroxisome abundance in *H. polymorpha*. Deletion of *DNM1* however resulted in one peroxisome /cell whereas WT control cells contained 3-4 peroxisomes. Life cell imaging confirmed that these single organelles ultimately divide during cytokinesis, similar as in the *S. cerevisiae* *vps1.dnm1* double mutant.

We showed that also in *H. polymorpha* Dnm1 has dual localization to mitochondria and peroxisomes. Life cell imaging revealed that fluorescent Dnm1p-GFP is present in multiple spots that fluctuate between peroxisomes and mitochondria.

Pex11 is a peroxisome membrane protein required for peroxisome proliferation. The number of peroxisomes in a cell can be altered by modulation of the Pex11 levels. To know if Pex11 is required for making the peroxisome extension in *dnm1* cells, we analyzed a *pex11.dnm1* double deletion. These cells have a single enlarged organelle, which does not form extensions to the developing bud. In *dnm1* cells Pex11p is present over the entire organelle surface, but concentrates during fission at the basis of the organelle extension. These findings indicate that Pex11 is important for developing peroxisome extensions/elongations prior to the Dnm1-mediated fission process.

In Chapter 4 we analyzed two other components of the mitochondrial fission machinery of *S. cerevisiae* for their role in peroxisome fission. In *S. cerevisiae* deletion of *MDV1*, *CAF4* or both, however, had only a minor effect on peroxisome numbers at peroxisome-inducing growth conditions, most likely related to the fact that Vps1 - and not Dnm1 - is the key player in peroxisome fission in this organism.

In contrast, in *H. polymorpha*, which lacks the *MDV1* paralog *CAF4*, deletion of *MDV1* led to a drastic reduction of peroxisome numbers. This phenotype was accompanied by a strong defect in mitochondrial fission. Hence, in *H. polymorpha* peroxisomes and mitochondria share the same organelle fission machinery.

Next, we showed that in WT *H. polymorpha* cells Dnm1-mCherry and GFP-Mdv1 co-localize in spots that associate with both peroxisomes and mitochondria. Furthermore, our data

indicate that Fis1 is essential to recruit Mdv1 to the peroxisomal and mitochondrial membrane. However, the punctuate localization of GFP-Mdv1 and the organelle fission is strictly dependent on the presence of Dnm1. In *dnm1* cells, GFP-Mdv1 is dispersed over the surface of peroxisomes and mitochondria. Also, in *H. polymorpha mdv1* or *fis1* cells the number of Dnm1-GFP spots is strongly reduced. These spots still associate to organelles, but are functionally inactive.

In Chapter 5 the role of membrane remodeling in the proliferation of peroxisomes is detailed. Division of peroxisomes needs membrane remodeling deduced from the finding that fission involves three distinct steps: membrane elongation, constriction and fission. We took advantage of the fact that in *H. polymorpha dnm1* cells organelle fission is blocked and only a single peroxisome is present, which forms a long extension prior to fission. The focal location of various key components of known protein complexes involved in organelle biogenesis, fission and inheritance in these cells were analyzed. A distinct localization of different proteins on different regions of the peroxisome membrane was observed. Pex14 and Pex25 were predominantly localized to the peroxisome extension during organelle fission. Inp1 was mainly localized on the organelle in the mother cell and Inp2 at the tip of the peroxisome extension in the daughter cell. These data show for the first time that different peroxisomal membrane proteins (PMPs) are differently distributed over the organelle surface, a mechanism that is most likely linked to allowing, completion of the organelle fission process.

To test whether Pex11 plays a role in the distribution of the PMPs, we analyzed the localizations of the above proteins in *dnm1.pex11* cells, which do not form peroxisome elongations. In these cells Pex25 and Pex14 no longer concentrate into spots, but are distributed over the entire organelle. Reintroduction of Pex11 in these cells led to the formation of the peroxisome elongations and Pex14-GFP that was distributed over the entire organelle subsequently re-concentrated into spots and was observed again localized on the newly formed peroxisome elongations.

These results clearly suggest that PMPs are organized in distinctive sub-domains on the membrane and Pex11 is required for this localization of these proteins. This is a completely novel function for Pex11, which was so far mainly implicated in peroxisome fission.